

Application No.: 10/733,741

Final Office Action dated: April 3, 2008

Attorney Docket No.: FNL0202US

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AUG 04 2008**Amendments to the Claims:**

This listing of claims will replace all prior versions and listing of claims in the application.

**Listing of Claims:****CLAIMS**

1. (previously presented) A method for automatic alignment of tilt series in an electron microscope, comprising:
  - applying markers to a sample to be imaged by the electron microscope;
  - providing a tilt series of images of the sample;
  - identifying a first set of candidate markers in each of the images in the tilt series;
  - attributing at least one probability parameter to each candidate marker in each image;
  - characterized in that the method further comprises:
    - selecting a second set as a subset of candidate markers from the first set of candidate markers on the basis of said at least one probability parameter;
    - projecting the candidate markers in the second set onto a sole image;
    - applying a fitting algorithm to determine a set of parallel straight lines or very elongate ellipses best fitting the candidate markers in the sole image to identify a third subset of candidate markers;
    - aligning the images in the tilt series on the basis of the third subset of identified candidate markers.
2. (original) A method according to Claim.1 in which the fitting algorithm used to determine the set of parallel straight lines comprises the Hough transformation.

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3. (original) A method according to Claim 1 in which the fitting algorithm used to determine the set of parallel straight lines or to determine a set of very elongate ellipses is constituted by the Generalized Hough transformation.

4. (original) A method according to Claim 1 in which, before identifying candidate markers in each of the images in the tilt series, a cross correlation process is applied to the images of the tilt series.

5. (previously presented) A method according to Claim 1 in which the probability parameter is derived from at least one of the quantities: size of the marker and local contrast of the marker.

6. (previously presented) A method for automatic alignment of tilt series in an electron microscope, comprising:

applying markers to a sample to be imaged by the electron microscope;  
providing a tilt series of images of the sample;  
identifying a first set of candidate markers in each of the images in the tilt series;  
attributing at least one probability parameter to each candidate marker in each image;  
characterized in that the method further comprises:  
selecting a second set as a subset of candidate markers from the first set of candidate markers on the basis of said at least one probability parameter;  
projecting the candidate markers in the second set onto a sole image;  
applying a fitting algorithm to determine a set of parallel straight lines or very elongate ellipses best fitting the candidate markers in the sole image to identify a third subset of candidate markers, the fitting algorithm including:

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deriving for each candidate marker in the second set a sine-shaped curve based on coordinates of the corresponding candidate marker, according to the Hough transformation;

deriving from the sine-shaped curves a number of histograms indicating, for each direction, the relation between the density of candidate markers and the line distance parameter according to the Hough transformation;

applying an entropy operation to each of the histograms, resulting in a set of entropy parameters, one entropy parameter for each histogram;

establishing the minimum value in the set of entropy parameters;

identifying the histogram corresponding to said minimum value as the one showing the highest degree of peak diversity;

selecting from the latter histogram a number of peaks; and

deriving from each peak position in the histogram the corresponding line distance parameter according to the Hough transformation.

7. (previously presented) A method according to Claim 2 in which the probability parameter is derived from at least one of the quantities: size of the marker and local contrast of the marker.

8. (previously presented) A method according to Claim 3 in which the probability parameter is derived from at least one of the quantities: size of the marker and local contrast of the marker.

9. (previously presented) A method according to Claim 4 in which the probability parameter is derived from at least one of the quantities: size of the marker and local contrast of the marker.